EXAMPLE 6

Inhibition of Dye Release by Free Hapten

The inhibitory effect of the free hapten to liposome lysis was also examined. As can be seen in FIG. 6, free hapten, DNP-Gly could effectively inhibit the dye release from the DOPE liposomes. The concentration of the free hapten which caused 50% inhibition was calculated to be 0.35 micromolar, which was equal to 14 picomole in 40 microliter of the preincubation medium. A non-hapten analog, Gly, had no effect on the dye release even at 1 millimolar concentration.

EXAMPLE 7

Inhibition of Dye Release by Free Antibody

Free anti-DNP IgG did not cause dye release even at 10 mg/ml. However, visible aggregation of the liposomes of DOPE or DOPC type was observed when free anti-DNP IgG was preincubated with the liposomes. Preincubation of the DOPE liposomes with free anti-DNP IgG, but not the normal IgG, caused inhibition of the dye release (FIG. 7). Fifty percent inhibition took place at the free antibody concentration of 0.5 mg/ml, which is equal to 2.5 microgram in a 5 microliter preincubation volume.

Materials

Dioleyl phosphatidylethanolamine (DOPE), dioeyl phosphatidylcholine (DOPC) and N-(dinitrophenylaminocaproyl)-phosphatidylethanolamine (DNP-cap-PE) were purchased from Avanti Polar Lipids, Inc. (Birmingham, Ala.). Calcein, N-(dinitrophenyl)-glycine (DNP-Gly), and glycine were purchased from Sigma Chemical Co. (St. Louis, Mo.). Other reagents were analytical grade.

ANTIBODY

Anti-DNP serum was prepared from rabbits immunized with DNP-derivatized bovine serum albumin (BSA) (Eisen et al., *Meth. Immunol. Immunochem.*, 1 351 (1967)) and was a generous gift from Dr. Stephen Kennel. IgG fractions were purified from the serum by the protein-A affinity column chromatography (Warr, *Antibody as a Tool: The Applications of Immunochemistry*, Marchalonis and Warr, eds., 59–96 (John Wiley and Sons, New York, 1982)) and stored in phosphate buffered saline (PBS) at −20° C. Antibody was attached to glass surface by adding 40 microliters of IgG solution at various concentrations to a spot 1.5 cm in diameter on a clean glass slide. After 20 minutes at room temperature, the slide was washed thoroughly with from 6 to 8 ml PBS, blotted to dryness except the spot, and immediately used for the subsequent experiment.

LIPOSOME PREPARATION

In routine experiments, DOPE or DOPC (8.8 micromole) DNP-cap-PE (1.2 micromole) and trace amount of hexadecyl [^{3}H] cholestanyl ether (final specific activity 5.7×10^9 cpm/mol) were mixed and evaporated free of solvent with a stream of N_2 gas. The dry lipid was vacuum dessicated for at least 30 minutes. One hundred microliters of PBS containing 4 micromoles calcein, pH 7.4, was added. The mixture was sonicated for 20 minutes at room temperature in a bath sonicator (Laboratory Supplies, Inc., Hicksville, N.Y.) until a uniform translucent liposome suspension was obtained. The liposome suspension was then chromatographed on a Biogel A50m column to remove any untrapped calcein. The liposome eluted with PBS in the void volume fractions and was detected by counting ^{3}H radioactivity, pooled and stored at 4° C.

LIPOSOME-ANTIBODY INTERACTIONS

Liposomes suspension (0.9 to 1.9 nanomole lipid in 5 to 45 microliters) was added to the spot on the glass slide which had previously been coated with IgG. After 20 minutes incubation in a moist chamber at room temperature, the glass slide was rinsed with 2 ml PBS to quantitatively transfer the liposomes into a quartz cuvette. The fluorescence was measured with a Perkin Elmer LS5 spectrofluorometer with lambda $_{ex}$=490 nm and lambda $_{em}$=520 nm. The total calcein fluorescence in the liposome was measured after the addition of sodium deoxycholate to a final concentration of 0.12%. The percent of dye release is defined as:

$$\%\ \text{release} = \frac{F - F_o}{F_t - F_o}$$

where F_o and F are the calcein fluorescence of the liposome sample before and after the interaction with the immobilized antibody, respectively. F_t is the total calcein fluorescence after releasing with the deoxycholate.

For the inhibition of dye release, free hapten in 40 microliters was added to the immobilized antibody on the glass slide and incubated for 20 minutes at room temperature before the addition of the liposomes. For the inhibition by free antibody, equal volumes of liposome and antibody were mixed and preincubated for 20 minutes at room temperature before being added to the glass slide.

90° C. LIGHT SCATTERING OF LIPOSOMES

In order to test for the liposome formation, sonicated lipids were diluted 100 fold in PBS. 90° light scattering was measured in a Perkin Elmer LS5 spectrofluorometer at lambda $_{ex}$=lambda $_{em}$=660 nm with a slit width of 3 nm.

ELECTRON MICROSCOPY

Liposome (0.75 micromole/ml) were negatively stained with 0.5% aqueous uranyl acetate and viewed in a Hitachi 600 electron microscope operated at 75 KV. The size of the liposomes was measured on photographically enlarged micrographs.

The method of the present invention can be applied to a wide variety of analytes. Antibodies, both polyclonal and monoclonal, can be raised using standard immunological techniques to numerous analytes. Other membrane-lytic techniques are also contemplated herein, for example, detection of enzymes or enzyme substrates using the assay of the present invention can be accomplished in a manner analogous to the detection of antigens or antibodies described supra.

In general, an enzyme substrate, which has been coupled to a suitable lipid (if necessary) is mixed with an H_{II} forming lipid such as DOPE to form stable liposomes containing a marker, such as the fluorescent dye. Interaction between these liposomes and the appropriate enzyme bound to a solid support causes lysis of the liposomes, releasing the fluorescent dye. Calibration of dye release is accomplished using standard enzyme or substrate concentrations and inhibition of dye release by unknown quantities of enzyme or substrate in a biologi-